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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 : C07F 9/58, A61K 31/675 A1

(11) International Publication Number: WO 98/28310

(43) International Publication Date: 2 July 1998 (02.07.98)

(21) International Application Number: PCT/EP97/07161

(22) International Filing Date: 17 December 1997 (17.12.97)

(30) Priority Data:
9626615.0 20 December 1996 (20.12.96) GB

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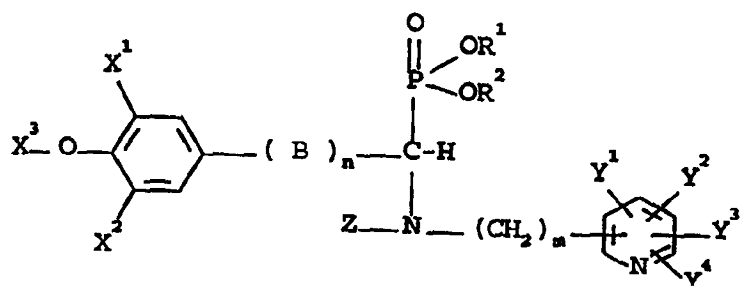
(81) Designated States: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(54) Title: PHARMACEUTICAL AMINOPHOSPHONIC ACID DERIVATIVES

(57) Abstract

Aminophosphonates alpha substituted by phenol groups, of formula (I) have lipoprotein(a) lowering activity.



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PHARMACEUTICAL AMINOPHOSPHONIC ACID DERIVATIVES

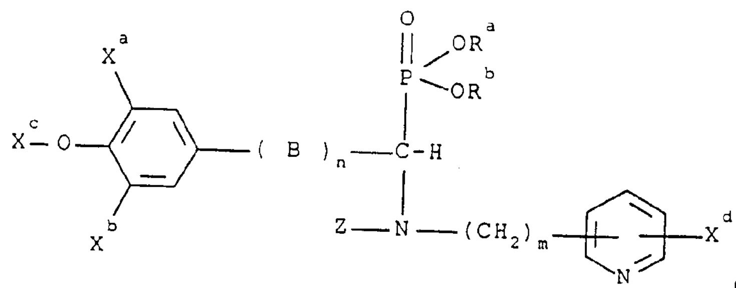
The present invention relates to novel aminophosphonate derivatives, processes for their preparations, pharmaceutical compositions containing them and their use in therapy, in particular for lowering lipoprotein(a) in plama and in tissues.

Lipoprotein(a) [Lp(a)] is a LDL-like lipoprotein where its major lipoprotein, apoB-100 is covalently linked to an unusual glycoprotein, apoprotein(a). Due to its structural similarity to plasminogen, apo(a) interfers with the normal physiological thrombosis-hemostasis process. The structural feature of Lp(a), where the LDL lipoprotein is linked to apo(a), is thought to be responsible for its atherogenic and thrombolytic activities.

Elevated levels of Lp(a) have been associated with the development of atherosclerosis, coronary heart disease, myocardial infarction, cerebral infarction, restenosis following balloon angioplasty and stroke. A recent epidemiologic study has provided the clinical proof of a positive correlation between plasma Lp(a) concentrations and the incidence of heart disease (see for instance: "Elevated Plasma Lipoprotein(a) and Coronary Heart Disease in Men Aged 55 Years and Younger"; A.G. Bostom, L. A. Cupples, J.L. Jenner, J.M. Ordovas, L.J. Seman, P.W.F. Wilson, E.J. Schaefer and W.P. Castelli; Journal of American Medical Association 1996, 276, p. 544-548).

Patients that have Lp(a) levels in excess of 20-30 mg/dl run a significantly increased risk of heart attacks and stroke. An effective therapy for lowering Lp(a) does not exist at present as potent hypocholesterolemic agents such as the HMGCoA reductase inhibitors do not affect Lp(a). Until recently, the only compound shown to lower Lp(a) was niacin. The high doses necessary for activity however entail unacceptable side-effects. There is therefore an unmet therapeutic need for agents that effectively reduce elevated levels of Lp(a).

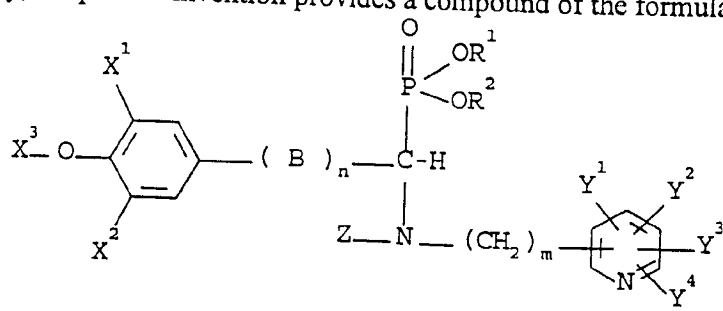
International application WO97/02037 (Symphar SA; SmithKline Beecham plc, published 23 January 1997), published after the priority date of the present application, describes a group of aminophosphonates alpha substitued by phenol groups of the formula (A):



(A)

in which X^a is H, $C_{(1-8)}$alkyl, hydroxy or $C_{(1-8)}$alkoxy; $X^b$is $C_{(1-8)}$alkyl or $C_{(1-8)}$alkoxy; X^c is H, $C_{(1-4)}$alkyl, or X^3O and one of the two other substituents X^a or X^b may form an alkylidene dioxy ring having from 1 to 4 carbon atoms; R^a and R^b which may be identical or different, are H or $C_{(1-6)}$alkyl; B is CH_2CH_2, CH=CH, or CH_2; n is zero or 1; Z is H or a $C_{(1-8)}$alkyl group; m is 0 or an integer from 1 to 5; X^d is H, or $C_{(1-8)}$alkyl, $C_{(1-8)}$alkoxy or halo; and the pyridyl ring is attached by the ring carbon α- or β- to the nitrogen (2- or 3-pyridyl). These have Lp(a) lowering activity. Compounds of formula (A) fall within scope of the generic disclosure of EP-A-0 559 079. This is directed towards aminophosphonates alpha substitued by phenol groups which are said to be of use in decreasing plasma cholesterol and blood peroxides. Compounds of formula (A) are characterised by having either no substituent (X^d is H) or a single substituent on the pyridyl ring. It has now been found that further substitution on the pyridyl ring provides compounds with an improved biological profile.

Accordingly, the present invention provides a compound of the formula (I):



(I)

in which:

X^1 and X^2, which may be the same or different, are H, a straight or branched $C_{(1-8)}$alkyl or $C_{(1-8)}$alkoxy group, a hydroxy group or a nitro group;

X^3 is H, a $C_{(1-4)}$alkyl group, X^3O and one of the two other substituents X^1 or X^2 may form a $C_{(1-4)}$alkylidene dioxy ring;

R^1 and R^2, which may be the same or different, are H, a straight or branched $C_{(1-6)}$alkyl group;

B is CH_2, CH_2-CH_2 or CH=CH;

n is zero or 1;

Z is H, or a straight or branched $C_{(1-8)}$alkyl group;
m is 0 or an integer from 1 to 5; and
Y^1, Y^2, Y^3 and Y^4, which may be the same or different, are H, a straight or branched $C_{(1-8)}$alkyl or $C_{(1-8)}$alkoxy group, a cyano, trifluoromethyl, nitro, hydroxy, hydroxymethyl, $C_{(1-4)}$ alkoxymethyl, amino, $C_{(1-4)}$alkylamino, $C_{(1-4)}$dialkylamino group, a halogen atom (F, Cl, Br, I), or any two adjacent Y^1, Y^2, Y^3 and Y^4 may form an optionally substituted $C_{(1-6)}$alkylidene or $C_{(1-4)}$alkylidenedioxy ring, with the proviso that at least two of the Y^1, Y^2, Y^3 and Y^4 groups are not H;
or a pharmaceutically acceptable salt thereof.

Preferably, X^1 is H, hydroxy, $C_{(1-4)}$alkyl or $C_{(1-4)}$alkoxy, preferably $C_{(1-3)}$alkyl or $C_{(1-3)}$alkoxy, more preferably hydrogen, hydroxy, methyl, methoxy or ethoxy.

Preferably, X^2 is $C_{(1-4)}$alkyl or $C_{(1-4)}$alkoxy, preferably $C_{(1-3)}$alkyl or $C_{(1-3)}$alkoxy, more preferably methyl, methoxy or ethoxy.

Preferably, X^1 and X^2 is each $C_{(1-4)}$alkyl, preferably $C_{(1-3)}$alkyl, or $C_{(1-4)}$alkoxy; or or one of X^1 and X^2 is $C_{(1-4)}$alkyl and the other is $C_{(1-4)}$alkoxy or $C_{(1-3)}$alkyl; or X^1 is hydroxy and X^2 is $C_{(1-4)}$alkyl or $C_{(1-4)}$alkoxy.

Preferred combinations of X^1 and X^2 include methoxy and methoxy, methoxy and methyl, ethoxy and methyl, methyl or t-butyl and methyl, ethoxy and ethoxy, hydroxy and methyl, and hydroxy and methoxy, respectively.

Preferably, X^3 is hydrogen or methyl.

A particularly preferred phenyl group is 4-hydroxy-3-methoxy-5-methylphenyl.

Preferably, $(B)_n$ is a direct bond.

Preferably, m is zero.

Preferably, R^1 and R^2 is each a $C_{(1-3)}$alkyl group, more preferably, a C_2 or C_3 alkyl group, in particular R^1 and R^2 is ethyl or isopropyl.

Preferably, Z is hydrogen.

Representative values for Y^1 to Y^4 include alkyl, for instance methyl or t-butyl, methoxy, chloro, hydroxy, hydroxymethyl or two adjacent substituents form an optionally substituted alkylidene or alkylidenedioxy ring having from 1 to 6 carbon atoms.

Preferably, Y^1 and Y^2 is each methyl, preferably as 2,6-substituents of the pyridyl ring, and Y^3 and Y^4 is each hydrogen,.

Preferably, the pyridyl ring is attached by the ring carbon β- to the nitrogen (3/5-pyridyl). A particularly preferred pyridyl ring is (2,6-dimethyl)pyrid-3-yl.

Pharmaceutically acceptable salts are well known in the art and include inorganic and organic salts, for instance salts with HCl, H_2SO_4, oxalic acid, maleic acid, sulfonic acid, etc..

Preferred compounds of formula (I) include:
Diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate; and
Diethyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate;
and pharmaceutically acceptable salts;
in particular:
(+)-diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate; and
pharmaceutically acceptable salts thereof, in particular, the hydrochloride salt.

Compounds of formula (I) are found to be effective in decreasing Lp(a) production by primary cultures of Cynomolgus monkey hepatocytes. The Lp(a) of these primates is similar in immunologic properties to human Lp(a) and occurs in an almost identical frequency distribution of plasma concentrations (see "Plasma Lipoprotein(a) Concentration is Controlled by Apolipoprotein(a) Protein Size and the Abundance of Hepatic Apo(a) mRNA in a Cynomolgus Monkey Model", N. Azrolan *et al*, J. Biol. Chem., 266, 13866-13872, 1991). The compounds of formula (I) are thus potentially useful for decreasing Lp(a) in man and thereby providing a therapeutic benefit.
Accordingly, in a further aspect, the present invention provides a compound of formula (I) or a pharmaceutically acceptable salt thereof for use in therapy, in particular as an Lp(a) lowering agent. Elevated plasma and tissue levels of lipoprotein(a) is associated with accelerated atherosclerosis, abnormal proliferation of

smooth muscle cells and increased thrombogenesis and expressed in disease states such as, for instance: coronary heart disease, peripheral artery disease : intermittent claudication, thrombosis, restenosis after angioplasty, extracranial carotid atherosclerosis, stroke and atherosclerosis occuring after heart transplant. Compounds of formula (I) may also be useful in treating inflammatory diseases and excessive wound healing.

For such therapeutic use, the compounds of the present invention will generally be administered in a standard pharmaceutical composition. Accordingly, in a further aspect, the present invention provides for a pharmaceutical composition comprising a compound of formaula (I) and a pharmaceutically acceptable excipient or carrier. Suitable excipients and carriers are well known in the art and will be selected with regard to the intended route of administration and standard pharmaceutical practice. For example, the compositions may be administered orally in the form of tablets containing such excipients as starch or lactose, or in capsule, ovules or lozenges either alone or in admixture with excipients, or in the form of elixirs or suspensions containing flavoring or coloring agents. They may be injected parenterally, for example, intravenously, intramuscularly or subcutaneously. For parenteral administration, they are best used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The choice of form for administration as well as effective dosages will vary depending, *inter alia*, on the condition being treated. The choice of mode of administration and dosage is within the skill of the art.

The compounds of formula (I) and their pharmaceutically acceptable salts which are active when given orally can be formulated as liquids, for example syrups, suspensions or emulsions or as solids for example, tablets, capsules and lozenges. A liquid formulation will generally consist of a suspension or solution of the compound or pharmaceutically acceptable salt in suitable liquid carrier(s) for example, ethanol, glycerine, non-aqueous solvent, for example polyethylene glycol, oils, or water with a suspending agent, preservative, flavoring or coloring agents. A composition in the form of a tablet can be prepared using any suitable pharmaceutical carrier(s) routinely used for preparing solid formulations. Examples of such carriers include magnesium stearate, starch, lactose, sucrose and cellulose. A composition in the form of a capsule can be prepared using routine encapsulation procedures. For example, pellets containing the active ingredient can be prepared using standard carriers and then filled into a hard gelatin capsule; alternatively, a dispersion or suspension can be prepared using any suitable pharmaceutical carrier(s), for example aqueous gums, celluloses,

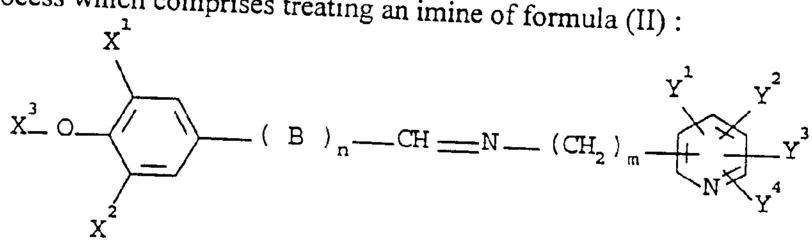
silicates or oils and the dispersion or suspension then filled into a soft gelatin capsule. Typical parenteral compositions consist of a solution or suspension of the compound or pharmaceutically acceptable salt in a sterile aqueous carrier or parenterally acceptable oil, for example polyethylene glycol, polyvinyl pyrrolidone, lecithin, arachis oil or sesame oil. Alternatively, the solution can be lyophilised and then reconstituted with a suitable solvent just prior to administration. A typical suppository formulation comprises a compound of formula (I) or a pharmaceutically acceptable salt thereof which is active when administered in this way, with a binding and/or lubricating agent such as polymeric glycols, gelatins or cocoa butter or other low melting vegetable or synthetic waxes or fats.

Preferably the composition is in unit dose form such as a tablet or capsule. Each dosage unit for oral administration contains preferably from 1 to 250 mg (and for parenteral administration contains preferably from 0.1 to 25 mg) of a compound of the structure (I) or a pharmaceutically acceptable salt thereof calculated as the free base.

The compounds of the invention will normally be administered to a subject in a daily dosage regimen. For an adult patient this may be, for example, an oral dose of between 1 mg and 500 mg, preferably between 1 mg and 250 mg, or an intravenous, subcutaneous, or intramuscular dose of between 0.1 mg and 100 mg, preferably between 0.1 mg and 25 mg, of the compound of the formula (I) or a pharmaceutically acceptable salt thereof calculated as the free base, the compound being administered 1 to 4 times per day.

Compounds of formula (I) may be prepared by processes well known in the art, for instance those previously described in WO 97/02037.

Thus, for instance, compounds of formula (I) in which Z is hydrogen may be prepared by a process which comprises treating an imine of formula (II) :



(II)

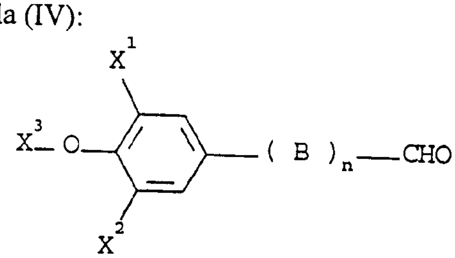
in which X^1, X^2, X^3, B, n, m, Y^1, Y^2, Y^3 and Y^4 are as previously defined; with a dialkyl phosphite of formula (III):

$$H\text{-}PO(OR^1)(OR^2) \quad \text{(III)}$$

in which R^1 and R^2 are as previously defined; or a trialkyl silyl derivative thereof, preferably the trimethyl silyl phosphite, or a metal thereof, for instance the sodium salt, formed *in situ* by treatment of the compound of formula (III) with a suitable base, for instance sodium hydride, ethoxide or methoxide.

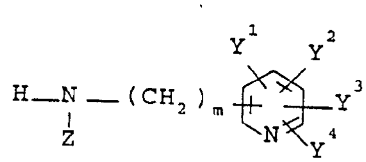
The reaction may be carried out in presence or absence of a catalyst. Suitable catalysts incude an amine such as diethylamine or triethylamine. The reaction may be carried out in presence or in absence of a solvent. Suitable solvents include petroleum ether, benzene, toluene, diethyl ether, tetrahydrofuran, 1,2-dimethoxyethane. Suitable reaction temperatures are in the range of 30 to 140°C.

The imine compound of formula (II) may be obtained by condensing an aldehyde compound of formula (IV):



(IV)

in which X^1, X^2, X^3, B and n are as previously defined; with a primary amine of formula (V):



(V)

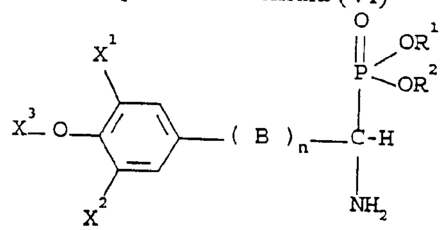
in which Z, m, Y^1, Y^2, Y^3 and Y^4 are as previously defined; under imine forming conditions.

Suitably, the condensation may be effected with or without a catalyst in a solvent such as ether, tetrahydrofuran, benzene, toluene or ethanol. Suitable catalysts include molecular sieve, an acid such as glacial acetic acid, p-toluenesulfonic acid, thionyl chloride, titanium tetrachloride, boron trifluoride etherate, or a base such as potassium carbonate. The reaction is suitably carried out in the range of 0°C to the boiling point of the solvent being used. For less reactive amines or aldehydes, the reaction may be usefully carried out in a Dean-Stark apparatus.

Compounds of formula (I) may also be prepared by a process which comprises treating equimolar amounts of an aldehyde of formula (IV) , an amine of formula (V) in which Z, m, Y^1, Y^2, Y^3 and Y^4 are as previously described; and a dialkyl

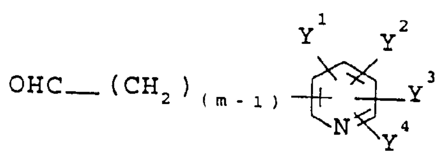
phosphite of formula (III), suitably in the presence of p-toluenesulfonic acid as a catalyst, in a hydrocarbon solvent such as petroleum ether, benzene, toluene or xylene, at a temperature between ambient room temperature and the boiling point of the solvent being used, and with concomitant elimination of water, for instance, by using a Dean-Stark apparatus.

Compounds of formula (I) in which m is not zero may also be prepared by a process which comprises treating a compound of formula (VI)



(VI)

in which X^1, X^2, X^3, B and n are as previously defined; with an aldehyde of formula (VII):



(VII)

in which m is an integer from 1 to 5 and Y^1, Y^2, Y^3 and Y^4 are as previously defined; under reductive amination conditions.

Suitable such conditions include carrying out the reaction in the presence of sodium cyanoborohydride in an alcoholic solvent, preferably methanol, at a pH between 3 to 6 and at a temperature between 0°C and 25°C.

A compound of formula (VI) may be obtained according to the process previously described for a compound of formula (I) from an aldehyde of formula (IV), an amine of formula (VIII)

$$A\text{-}NH_2 \quad \text{(VIII)}$$

in which A is a protecting group which can be removed by hydrogenolysis, for instance an α substituted benzyl or benzyloxycarbonyl and a phosphite of structure (III). This forms an intermediate which is then subjected to hydrogenolysis according to standard conditions, to give a compound of formula (VI).

It will be appreciated that the aminophosphonate ester of formula (I) have a basic centre and can form salts, for instance with inorganic acids such as HCl, H_2SO_4 and

with organic acids such as oxalic acid, maleic acid, sulfonic acids, etc... All these salts are integral part of this invention.

Compounds of structure (I) are racemates as they have at least one chiral center which is the carbon atom in position alpha to the phosphonate group. The compounds of formula (I) therefore exist in the two enantiomeric forms. The racemic mixtures (50% of each enantiomer), the pure enantiomers and other mixtures thereof all form part of the present invention. Mixtures of enantiomers, including racemates, may be resolved into its constituent enantiomers according to procedures well known in the art, including for instance, chiral chromatography. Unless otherwise indicated, the physical constants and biological data given for compounds of structure (I) refer to racemates.

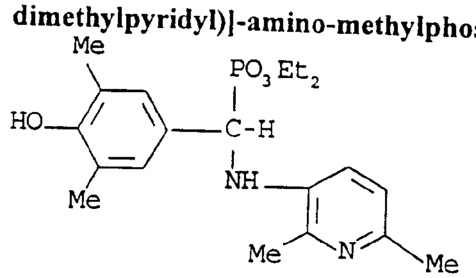
The structure of compounds of formula (I) described in the following Examples was established by their infrared (IR), mass (MS) and nuclear magnetic resonance (NMR) spectra. The purity of the compounds was checked by thin layer, gas liquid or high performance liquid chromatography.

The invention is further described in the following examples which are intended to illustrate the invention without limiting its scope.

The abbreviations used in this application are the following :
In the tables, n is normal, i is iso, s is secondary and t is tertiary. In the description of the NMR spectra, respectively 's' is singlet, 'd' is doublet, 'dd' is double doublet, 't' is triplet and 'm' is multiplet. TsOH is p-toluenesulfonic acid monohydrate. The temperatures were recorded in degrees Celsius and the melting points are not corrected.

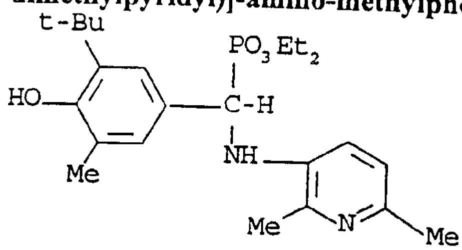
Examples

Example 1 - Diethyl α-(4-hydroxy-3,5-dimethylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



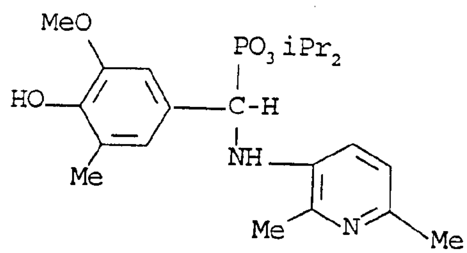
A mixture of 1.11 g (7.4 mmol) of 4-hydroxy-3,5-dimethylbenzaldehyde, 0.9 g (7.4 mmol) of 3-amino-2,6-dimethylpyridine, 3.05g (22 mmol) diethylphosphite and ca 5 mg TsOH dissolved in 20 ml toluene contained in a flask connected to a Dean Stark apparatus was refluxed for 7 h. The solvent and the excess of diethylphosphite were evaporated to give a yellow oil which was purified by column chromatography (SiO_2, 95/5 $CHCl_3$/MeOH) to give 0.38 g (21%) of an oil which slowly solidified.
MS (m/e) = 392 : M^+, 255 (100%) : $M^+ - PO_3Et_2$
NMR ($CDCl_3$):δ = 7.0 (d, J = 2 Hz, 2H): aromatic H, substituted phenyl; 6.73 and 6.61 (2m, 1H each): aromatic H, 3-pyridyl; 5.3 (1H) : OH; 4.55 (dd, J = 7 and 22 Hz, 1H): CH-PO_3Et_2; 4.49 (m, 1H): N-H; 4.18 to 3.65 (m, 4H): P-O-CH_2-CH_3; 2.49 and 2.36 (2s, 6H total): Py-CH_3; 2.2 (1s, 6H): Ph-CH_3; 1.29 and 1.15: (2t, J=7Hz, 6H total): P-O-CH_2-CH_3

Example 2 - Diethyl α-(3-tert-butyl-4-hydroxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



A mixture of 1.42 g (7.4 mmol) of 3-tert-butyl-4-hydroxy-5-methyl-benzaldehyde, 0.9 g (7.4 mmol) of 3-amino-2,6-dimethylpyridine, 3.05g (22 mmol) diethylphosphite and ca 5 mg TsOH dissolved in 20 ml toluene contained in a flask connected to a Dean Stark apparatus was refluxed for 7 h. The solvent and the excess of diethylphosphite were evaporated and the residue was purified by column chromatography (SiO_2, 95/5 $CHCl_3$/MeOH) and recrystallization to give 0.89 g (21%) of a solid, mp = 139-141°C.
MS (m/e) = 434 : M^+, 297 (100%) : $M^+ - PO_3Et_2$
NMR ($CDCl_3$): δ = 7.15 and 7.02 (2m, 2H): aromatic H, substituted phenyl; 6.74 and 6.62 (2m, 1H each): aromatic H, 3-pyridyl; 5.15 (1H) : OH; 4.59 (dd, J = 7 and 23 Hz, 1H): CH-PO_3Et_2; 4.47 (m, 1H): N-H; 4.18 to 3.65 (m, 4H): P-O-CH_2-CH_3; 2.49 and 2.36 (2s, 6H total): Py-CH_3; 2.18 (1s, 3H): Ph-CH_3; 1.39 (s, 9H): t-Bu; 1.29 and 1.13: (2t, J=7Hz, 6H total): P-O-CH_2-CH_3

Example 3 - Diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



A mixture of 4.0 g (24 mmol) of 4-hydroxy-3-methoxy-5-methylbenzaldehyde and 2.94 g (24 mmol) of 3-amino-2,6-dimethylpyridine dissolved in 40 ml toluene and a catalytic amount of p-toluenesulfonic acid (ca. 5 mg) contained in a flask connected to a Dean Stark apparatus was refluxed for 7 h. The solution was evaporated to dryness to give 6.5 g (100%) of an orange oil which was used directly for the next step. Diisopropyl phosphite (5.84 g, 35 mmol) was added to 3.8 g (14 mmol) of the crude imine dissolved in 40 ml toluene and the mixture was refluxed for 7 h. A further amount of diisopropyl phosphite (2.34 g, 14 mmol) was added and the mixture was refluxed for 2 more hours (total reaction time : 9 h). The solvent and the excess of diisopropyl phosphite were evaporated and the residue was purified by column chromatography (SiO_2, 95/5 $CHCl_3$/MeOH) and recrystallization (petroleum ether/CH_2Cl_2) to give 1.48 g (24%) of a tan solid, mp = 138-139°C. A further recrystallisation from a t-butyl methyl ether/CH_2Cl_2 mixture yielded a light yellow solid of analytical purity, mp=159-160°C.

Elemental analysis: $C_{22}H_{33}N_2O_5P$

% Calc.	C 60.54	H 7.62	N 6.47	P7.27
% Found	C 60.45	H 7.76	N 6.35	P7.09

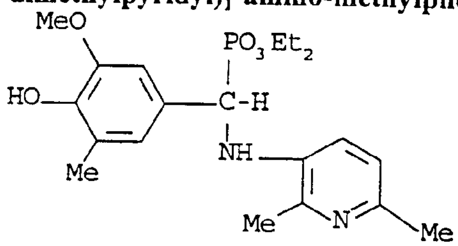
MS (m/e) = 436 : M^+, 271 (100%) : M^+ - PO_3iPr_2

NMR ($CDCl_3$):δ = 6.80 and 6.73 (2m, 1H each): aromatic H, 3-pyridyl; 6.6 (m, 2H): aromatic H, substituted phenyl; 5.7 (1H) : O<u>H</u>; 4.65 and 4.47(m, 2H): P-O-C<u>H</u>-Me_2; 4.5 (2 overlapped m, 2H): C<u>H</u>-PO_3iPr_2 and N-<u>H</u>; 3.85 (s, 3H): OC<u>H</u>$_3$; 2.50 and 2.37 (2s, 6H total): Py-C<u>H</u>$_3$; 2.22 (1s, 3H): Ph-C<u>H</u>$_3$; 1.32, 1.29, 1.23 and 1.01: (4d, J=7Hz, 12H total): P-O-CH-(C<u>H</u>$_3$)$_2$

This compound may also be prepared in 1,2-dimethoxyethane (DME). The imine (8.1g, 0.03 mol) was dissolved in 10 ml DME and diisopropyl phosphite (7.5 g, 0.045 mol) was added and the resulting mixture was refluxed overnight. DME was evaporated under vacuum to give a material which was purified by column chromatography (95/5 $CHCl_3$/MeOH); the collected fractions gave after trituration in petroleum ether and two recrystallisations in CH_2Cl_2/MTBE 6.9 g (52%) of pure title compound, mp = 159-160°C.

Alternately the reaction may be carried out neat (without solvent) in the phosphite reagent. To the crude imine (8.1g, 0.03 mol) was added diisopropyl phosphite (7.5 g, 0.045 mol) and the homogenous brown mixture was heated at 120°C for 2 hours. The oily reaction mixture was diluted in chloroform and extracted with a saturated bicarbonate solution. The dried organic phase was concentrated and triturated in petroleum ether to remove the excess of HPO_3iPr_2 : a pasty solid was obtained. Column chromatography (95/5 $CHCl_3$/MeOH) and recrystallisation gave 6.5g (50%) of the title compound, mp = 159-160°C.

Example 4 - Diethyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate

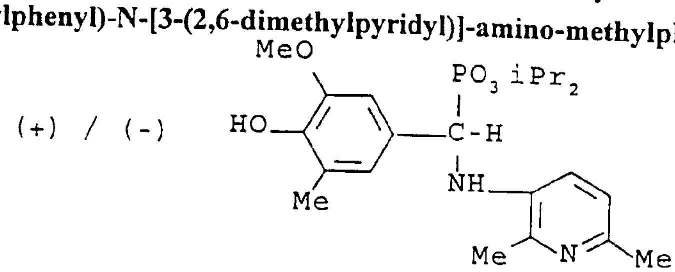


As described in Example 3, the imine (3.8 g, 14 mmol) obtained by condensing 4-hydroxy-3-methoxy-5-methylbenzaldehyde with 3-amino-2,6-dimethylpyridine was reacted with diethyl phosphite (5.82 g, 42 mmol) in 40ml toluene at reflux temperature for 9h to give 1.38 g (24%) of the title compound as a white solid, mp = 145-147°C.

MS (m/e) = 408 : M^+, 271 (100%) : M^+ - PO_3Et_2

NMR ($CDCl_3$):δ = 6.82 and 6.76 (2m, 1H each): aromatic H, 3-pyridyl; 6.6 (m, 2H): aromatic H, substituted phenyl; 5.7 (1H) : O<u>H</u>; 4.62-4.47 (2 overlapped m, 2H): C<u>H</u>-PO_3Et_2 and N-<u>H</u>; 4.18 to 3.7 (m, 4H): P-O-C<u>H</u>$_2$-CH_3; 3.86 (s, 3H): OC<u>H</u>$_3$; 2.52 and 2.39 (2s, 6H total): Py-C<u>H</u>$_3$; 2.24 (1s, 3H): Ph-C<u>H</u>$_3$; 1.31 and 1.19: (2t, J=7Hz, 6H total): P-O-CH_2-C<u>H</u>$_3$

Example 5 - Enantiomers of diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



The enantiomers of a racemic mixture were separated by simulated moving bed chromatography using eight columns packed with 30 g of Chiralpak AD and hexane/ethanol (9/1) as the eluent. 42 g of the racemic mixture was processed to give

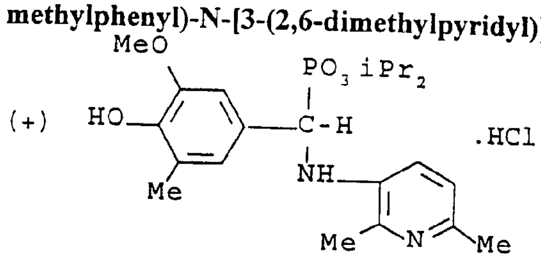
after trituration with diethyl ether 16.1 g of the faster eluting enantiomer ([α]D25 +14.0° (c = 1.0 EtOH), mp = 123-124°C, optical purity = 98.5%) and 15.2 g of the slower eluting enantiomer ([α]D25 -13.1° (c = 1.0 EtOH), mp = 120-122°C, optical purity = 97.5%)

The structures of both enantiomers were confirmed by NMR, IR and MS spectroscopies and elemental analyses.

Elemental analysis: $C_{22}H_{33}N_2O_5P$

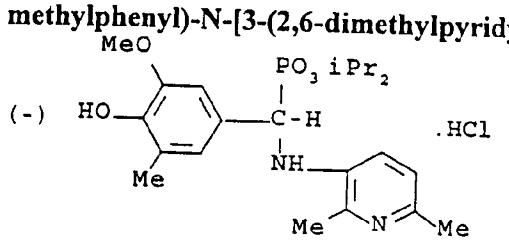
% Calc.	C 60.54	H 7.62	N 6.47
(+) Enantiomer	C 60.57	H 7.98	N 6.40
(-) Enantiomer	C 60.45	H 7.94	N 6.32

Example 6 - Hydrochloride salt of (+)diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



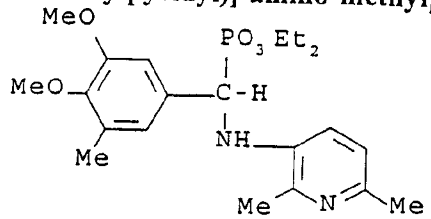
(+)Diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate (1.5 g) was dissolved in 30 ml EtOH and cooled in an ice bath. A solution of HCl in Et_2O (1M, 3.45ml) was added, after stirring for 10 min the mixture was concentrated under reduced pressure. The residue was crystallized from ethyl acetate to give 1.25 g of a white solid, [α]D25 +45.6° (c = 0.535 EtOH), optical purity 99.9%.

Example 7 - Hydrochloride salt of (-)diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



(-)Diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate (1.11 g) was dissolved in 25 ml EtOH and cooled in an ice bath. A solution of HCl in Et_2O (1M, 2.54ml) was added, after stirring for 10 min the mixture was concentrated under reduced pressure. The residue was crystallized from ethyl acetate to give 0.98 g of a white solid, [α]D25 -39.3° (c = 0.595 EtOH), optical purity 94.0%.

Example 8 - Diethyl α-(3,4-dimethoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



Methyl iodide (50 ml, 113.8 g, 0.8 mol) was added to a mixture containing 16.6 g (0.1mol) 4-hydroxy-3-methoxy-5-methyl-benzaldehyde, 55.2g (0.4mol) potassium carbonate in 90 ml methyl ethyl ketone and the resulting mixture was refluxed for 5 h. The solvent was evaporated on a rotary evaporator and the residue was partitioned between 100 ml H_2O and 100 ml CH_2Cl_2. The aqueous phase was further extracted by three 100 ml portions of CH_2Cl_2, the combined organic phases were dried over $MgSO_4$ and evaporated to give an orange oil weighing 18 g (100%). NMR ($CDCl_3$) : α =9.83 (1H, CHO), 7.3 (2H, aromatic H), 3.92 and 3.90 (6H, OMe) and 2.33 (3H, Me).

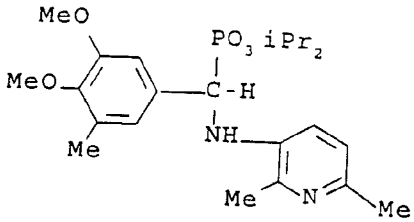
A mixture of 1.62 g (9 mmol) of 3,4-dimethoxy-5-methylbenzaldehyde obtained as described above and 1.1 g (9 mmol) of 3-amino-2,6-dimethylpyridine dissolved in 25 ml toluene and a catalytic amount of p-toluenesulfonic acid (ca. 1 mg) contained in a flask connected to a Dean Stark apparatus was refluxed for 8 h. The solution was evaporated to dryness to give 2.56 g (100%) of an orange oil which was used directly for the next step.

Diethyl phosphite (3.73 g, 27 mmol) was added to 2.56 g (9 mmol) of the crude imine dissolved in 25 ml toluene and the mixture was refluxed for 8 h. The solvent and the excess of diethyl phosphite were evaporated and the residue was purified by column chromatography (SiO_2, 95/5 $CHCl_3$/MeOH) to give 2.7 g (71%) of a yellow oil.

MS (m/e) = 423 : M^++1, 286 (100%) : M^++1 - PO_3Et_2

NMR ($CDCl_3$): δ = 6.83 (m, 2H): aromatic H, substituted phenyl; 6.75 and 6.60 (2d, 1H each): aromatic H, 3-pyridyl; 4.62-4.47 (2 overlapped m, 2H): C<u>H</u>-PO_3Et_2 and N-<u>H</u>; 4.18 to 3.7 (m, 4H): P-O-C<u>H</u>$_2$-CH_3; 3.83 and 3.78 (2s, 6H): OCH_3; 2.51 and 2.39 (2s, 6H total): Py-CH_3; 2.24 (1s, 3H): Ph-CH_3; 1.30 and 1.16: (2t, J=7Hz, 6H total): P-O-CH_2-C<u>H</u>$_3$

Example 9 - Diisopropyl α-(3,4-dimethoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate

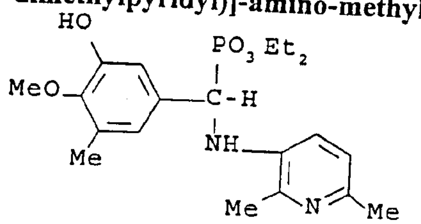


As described in Example 8, the imine (2.56 g, 9 mmol) obtained by condensing 3,4-dimethoxy-5-methylbenzaldehyde with 3-amino-2,6-dimethylpyridine was reacted with diisopropyl phosphite (4.49 g, 27 mmol) in 25ml toluene at reflux temperature for 9h to give 2.4 g (59%) of the title compound as a yellow oil, after purification by column chromatography (95/5 CH_2Cl_2/MeOH).

MS (m/e) = 451 : M^++1, 286 (100%) : M^+ - PO_3iPr_2

NMR ($CDCl_3$): δ = 6.81 (m, 2H): aromatic H, substituted phenyl; 6.75 and 6.65 (2m, 1H each): aromatic H, 3-pyridyl; 4.65 and 4.50(m, 2H): P-O-CH-Me_2; 4.5 (2 overlapped m, 2H): C<u>H</u>-PO_3iPr_2 and N-<u>H</u>; 3.82 and 3.76 (2s, 6H): OC<u>H</u>$_3$; 2.50 and 2.38 (2s, 6H total): Py-C<u>H</u>$_3$; 2.23 (1s, 3H): Ph-C<u>H</u>$_3$; 1.32, 1.29, 1.23 and 1.01: (4d, J=7Hz, 12H total): P-O-CH-$(CH_3)_2$

Example 10 - Diethyl α-(3-hydroxy-4-methoxy-5-methylphenyl)- N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



Anhydrous aluminum chloride(5.3 g, 40 mmol) was suspended under nitrogen in a solution of 4-hydroxy-3-methoxy-5-methylbenzaldehyde (6 g, 36 mmol) in 40 ml dichloromethane. Pyridine (12.8 ml, 160 mmol) was added dropwise while stirring and cooling to maintain the temperature between 30 and 35°C and the resulting orange solution was heated to reflux for 24h. After cooling the reaction mixture was hydrolyzed with a 10% HCl solution until pH 1-2. The resulting two phases were separated, the dichloromethane phase was discarded and the aqueous phase was extracted with three 40ml portions of diethyl ether. Evaporation of the dried ether phase gave 5.5g (100%) of a beige solid which was identified as 3,4-dihydroxy-5-methylbenzaldehyde.

Methyl iodide (5.6 ml, 12.83 g, 90 mmol) was added to a mixture of 3,4-dihydroxy-5-methylbenzaldehyde (5.5 g, 36 mmol) and lithium carbonate (6.68 g, 90 mmol) in N,N-dimethylformamide (90 ml) and the resulting mixture was heated to 55°C for 15h. Another portion of methyl iodide (2 ml) was added and the mixture was kept at 55°C for a further 4 h. The reaction mixture was poured into a mixture of 450 ml

water and 10 ml 37% HCl, the aqueous phase was extracted with three portions of 150 ml diethyl ether. The solvent was evaporated and the residue was purified by column chromatography to give 2.8 g (47%) of an oil identified as 3-hydroxy-4-methoxy-5-methylbenzaldehyde. MS (m/e) = 166 (100%): M^+, 151: M^+ - Me; NMR ($CDCl_3$) : δ =9.85 (1H, CHO), 7.32-7.28 (2H, aromatic H), 5.95 (1H, OH), 3.88 (3H, OMe) and 2.38 (3H, Me).

A mixture of 2.0 g (12 mmol) of 3-hydroxy-4-methoxy-5-methylbenzaldehyde obtained as described above and 1.47 g (12 mmol) of 3-amino-2,6-dimethylpyridine dissolved in 25 ml toluene and a catalytic amount of p-toluenesulfonic acid (ca. 1 mg) contained in a flask connected to a Dean Stark apparatus was refluxed for 4 h. The solution was evaporated to dryness to give 3.25 g (100%) of an brown solid which was used directly for the next step.

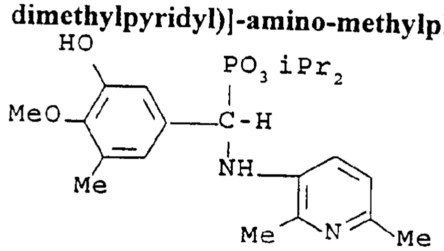
Diethyl phosphite (2.48 g, 18 mmol) was added to 1.63 g (6 mmol) of the crude imine dissolved in 25 ml toluene and the mixture was refluxed for 16 h. The solvent and the excess of diethyl phosphite were evaporated and the residue was purified by column chromatography (SiO_2, 95/5 CH_2Cl_2/MeOH) to give 0.9 g (37%) of a white solid, mp = 141-142°C after trituration in t-butyl methyl ether.

MS (m/e) = 409 : M^++1, 272 (100%) : M^++1- PO_3Et_2

NMR ($CDCl_3$):

δ = 8.0 (broad peak, 1H) : O<u>H</u>; 6.82 and 6.74 (2m, 2H): aromatic H, substituted phenyl, 6.75 and 6.58 (2m, 1H each): aromatic H, 3-pyridyl, 4.56 (dd, J =7 and 24Hz, 1H) C<u>H</u>-PO_3Et_2; 4.43 (dd, J =7 and 10Hz, 1H): N-<u>H</u>; 4.16 to 3.71 (m, 4H): P-O-C<u>H</u>$_2$-CH_3; 3.80 (s, 3H): OC<u>H</u>$_3$; 2.39 (1s, 3H): Ph-C<u>H</u>$_3$; 2.28 (1s, 6H total): Py-C<u>H</u>$_3$; 1.29 and 1.16: (2t, J=7Hz, 6H total): P-O-CH_2-C<u>H</u>$_3$

Example 11 - Diisopropyl α-(3-hydroxy-4-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate

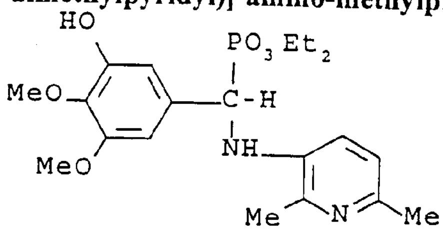


Diisopropyl phosphite (2.48 g, 18 mmol) was added to 1.63 g (6 mmol) of the crude imine dissolved in 25 ml toluene and the mixture was refluxed for 16 h. The solvent and the excess of diisopropyl phosphite were evaporated and the residue was purified by column chromatography (SiO_2, 95/5 CH_2Cl_2/MeOH) to give 1.1 g (42%) of a white solid, mp = 168-169°C after trituration in t-butyl methyl ether.

MS (m/e) = 436 : M^+, 271 (100%) : M^+ - PO_3iPr_2

NMR ($CDCl_3$): δ = 7.9 (broad peak, 1H) : O<u>H</u>; 6.83 and 6.74 (m, 2H): aromatic H, substituted phenyl; 6.74 and 6.58 (2d, 1H each): aromatic H, 3-pyridyl; 4.66 and 4.47 (2m, 2H): P-O-C<u>H</u>-Me_2; 4.54 -4.45 (2 overlapped m, 2H): C<u>H</u>-PO_3iPr_2 and N-<u>H</u>; 3.79 (s, 3H): OCH_3; 2.38 (1s, 3H): Ph-CH_3; 2.29 and 2.27 (2s, 6H total): Py-CH_3; 1.31, 1.29, 1.22 and 1.01: (4d, J=7Hz, 12H total): P-O-CH-$(CH_3)_2$

Example 12 - Diethyl α-(4,5-dimethoxy-3-hydroxyphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



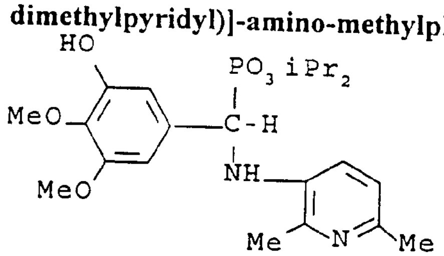
A mixture of 1.5 g (8 mmol) of 4,5-dimethoxy-3-hydroxybenzaldehyde and 0.98 g (8 mmol) of 3-amino-2,6-dimethylpyridine dissolved in 25 ml toluene and a catalytic amount of p-toluenesulfonic acid (ca. 1 mg) contained in a flask connected to a Dean Stark apparatus was refluxed for 16 h. The solution was evaporated to dryness to give 2.2 g (100%) of an oil which was used directly for the next step.

Diethyl phosphite (1.66 g, 12 mmol) was added to 1.15 g (4 mmol) of the crude imine dissolved in 25 ml toluene and the mixture was refluxed for 16 h. The solvent and the excess of diethyl phosphite were evaporated and the residue was purified by column chromatography (SiO_2, 95/5 CH_2Cl_2/MeOH) to give 0.52 g (30%) of a white solid, mp = 134-136°C.

MS (m/e) = 425 : M^++1, 288 (100%) : M^++1 - PO_3Et_2

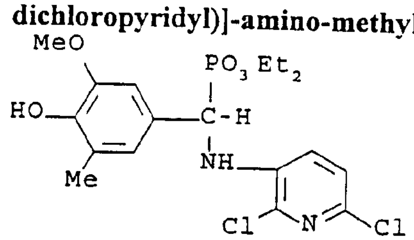
NMR ($CDCl_3$): δ = 7.2 (broad peak, 1H) : O<u>H</u>; 6.76 and 6.60 (2d, 1H each): aromatic H, 3-pyridyl; 6.64 and 6.57 (m, 2H): aromatic H, substituted phenyl; 4.57 (dd, J = 7 and 24Hz, 1H): C<u>H</u>-PO_3Et_2; 4.47 (dd, 1H): N-<u>H</u>; 4.18 to 3.76 (m, 4H): P-O-CH_2-CH_3; 3.87 and 3.84 (2s, 6H total): OCH_3; 2.39 and 2.38 (2s, 6H total): Py-CH_3; 1.30 and 1.19: (2t, J=7Hz, 6H total): P-O-CH_2-CH_3

Example 13 - Diisopropyl α-(4,5-dimethoxy-3-hydroxyphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



As described in Example 12, the imine (1.15 g, 4 mmol) obtained by condensing 4,5-dimethoxy-3-hydroxybenzaldehyde with 3-amino-2,6-dimethylpyridine was reacted with diisopropyl phosphite (2.0 g, 12 mmol) in 25ml toluene at reflux temperature for 16 h to give 0.5 g (28%) of the title compound as a solid, mp = 157-159°C after purification by column chromatography (95/5 CH_2Cl_2/MeOH).
MS (m/e) = 452 : M^+, 287 (100%) : M^+ - PO_3iPr_2
NMR ($CDCl_3$): δ = 6.9 (broad peak, 1H) : O<u>H</u>; 6.76 and 6.59 (2d, 1H each): aromatic H, 3-pyridyl; 6.64 and 6.57 (m, 2H): aromatic H, substituted phenyl; 4.69 and 4.51 (m, 2H): P-O-C<u>H</u>-Me_2; 4.5 (2 overlapped m, 2H): C<u>H</u>-PO_3iPr_2 and N-<u>H</u>; 3.86 and 3.85 (2s, 6H total): OCH_3; 2.41 and 2.38 (2s, 6H total): Py-CH_3; 1.33, 1.29, 1.23 and 1.04: (4d, J=7Hz, 12H total): P-O-CH-$(CH_3)_2$

Example 14 - Diethyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dichloropyridyl)]-amino-methylphosphonate

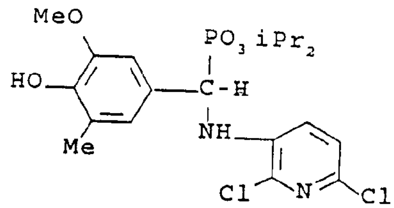


3-Amino-2,6-dichloropyridine (mp = 118-120°C) was obtained in quantitative yield by reacting 3-nitro-2,6-dichloropyridine with a mixture of reduced iron in aqueous acetic acid.

A mixture of 1.66 g (10 mmol) of 4-hydroxy-3-methoxy-5-methyl-benzaldehyde and 1.63 g (10 mmol) of 3-amino-2,6-dichloropyridine dissolved in 40 ml toluene and a catalytic amount of p-toluenesulfonic acid (ca. 1 mg) contained in a flask connected to a Dean Stark apparatus was refluxed for 16 h.

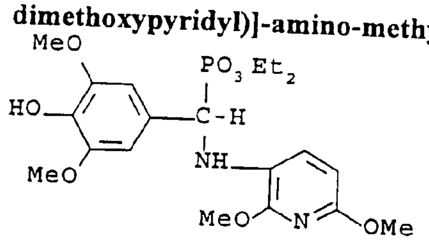
Diethyl phosphite (3.45 g, 25 mmol) was added to the toluene solution of the crude imine and the mixture was refluxed for 7 h. The solvent and the excess of diethyl phosphite were evaporated and the residue was purified by column chromatography (SiO_2, 95/5 CH_2Cl_2/MeOH) to give 0.52 g (30%) of a yellow solid.
MS (m/e) = 448 : $M^+(^{35}Cl)$, 311 (100%) : $M^+(^{35}Cl)$ - PO_3Et_2
NMR ($CDCl_3$): δ = 6.98 and 6.72 (2d, 1H each): aromatic H, 3-pyridyl; 6.77 (m, 2H): aromatic H, substituted phenyl; 5.71 (1H) : O<u>H</u>; 5.36 (dd, J = 7 and 10Hz, 1H): N-<u>H</u>; 4.53 (dd, J = 7 and 24Hz, 1H): C<u>H</u>-PO_3Et_2; 4.18 to 3.73 (m, 4H): P-O-CH_2-CH_3; 3.86 (s, 3H): OCH_3; 2.23 (1s, 3H): Ph-CH_3; 1.31 and 1.20: (2t, J=7Hz, 6H total): P-O-CH_2-CH_3

Example 15 - Diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dichloropyridyl)]-amino-methylphosphonate



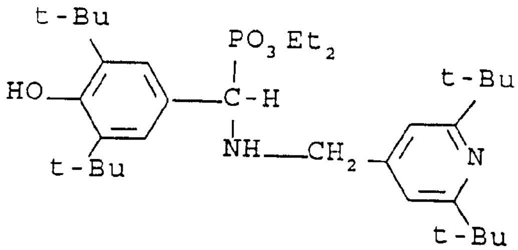
The process described in example 14 was followed using diiisopropyl phosphite as reagent to give the title compound as a white solid, mp = 124-125°C.
MS (m/e) = 476 : $M^+(^{35}Cl)$, 311 (100%) : $M^+(^{35}Cl)$ - PO_3iPr_2
NMR ($CDCl_3$): δ = 6.98 and 6.72 (2d, 1H each): aromatic H, 3-pyridyl; 6.77 (m, 2H): aromatic H, substituted phenyl; 5.71 (1H) : O<u>H</u>; 5.36 (dd, J = 7 and 10Hz, 1H): N-<u>H</u>; 4.67 and 4.50 (2m, 2H total): P-O-C<u>H</u>-Me_2; 4.5 (overlapped m, 1H): C<u>H</u>-PO_3iPr_2; 3.86 (s, 3H): $OC\underline{H}_3$; 2.23 (1s, 3H): Ph-$C\underline{H}_3$; 1.34, 1.31, 1.23 and 1.06: (4d, J=7Hz, 12H total): P-O-CH-$(C\underline{H}_3)_2$

Example 16 - Diethyl α-(3,5-dimethoxy-4-hydroxyphenyl)-N-[3-(2,6-dimethoxypyridyl)]-amino-methylphosphonate



The imine (0.60 g, 2 mmol) obtained by condensing 3,5-dimethoxy-4-hydroxy-benzaldehyde with 3-amino-2,6-dimethoxypyridine was reacted with diethyl phosphite (0.52 g, 4 mmol) in 25ml toluene at reflux temperature for 5h to give 0.34 g (40%) of the title compound as a brown oil, after purification by column chromatography (98/2 $CHCl_3$/MeOH).
MS (m/e) = 456 : M^+, 319 : M^+- PO_3Et_2
NMR ($CDCl_3$): δ = 6.68 (d, J = 2Hz, 2H): aromatic H, substituted phenyl; 6.56 and 6.07 (2d, J = 8Hz, 2H): aromatic H, 3-pyridyl; 4.53 (d, J = 23Hz, 1H): C<u>H</u>-PO_3Et_2; ca 4.0 (overlapped m): N<u>H</u>; 4.18 to 3.73 (m, 4H): P-O-$C\underline{H}_2$-CH_3; 3.98 and 3.81 (2s, 3H each): pyridyl-$OC\underline{H}_3$; 3.86 (1s, 6H): phenyl-$OC\underline{H}_3$; 1.29 and 1.19: (2t, J=7Hz, 6H total): P-O-CH_2-CH_3

Example 17 - Diethyl α-(3,5-di-tert-butyl-4-hydroxyphenyl)-N-[4-(2,6-di-tert-butylpicolyl)]-amino-methylphosphonate



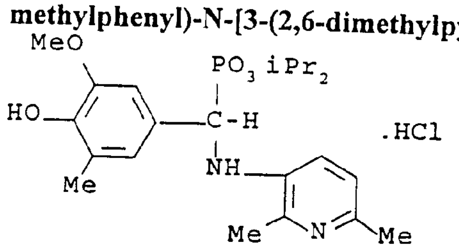
2,6-Di-tert-butylpyridine-4-carboxaldehyde was obtained by oxidation of 2,6-di-tert-butyl-4-methylpyridine with excess selenium dioxide in acetic acid at reflux temperature.

Diethyl α-(3,5-di-tert-butyl-4-hydroxyphenyl)-aminomethylphosphonate (1.67 g, 4.5 mmol) and 2,6-di-tert-butylpyridine-4-carboxaldehyde(1.8 g, 8.2 mmol) in 25 ml of MeOH were reacted with $NaBH_3CN$ (0.85 g, 13 mmol) for 4h. After neutralisation with diluted HCl the reaction mixture was extracted with CH_2Cl_2 and purified by column chromatography on silicagel (CH_2Cl_2 /MeOH) to yield 1.1g (43%) of the title compound; mp = 132-137°C;

MS (m/e) = 573 : M^+, 436 : M^+- PO_3Et_2

NMR ($CDCl_3$): δ = 7.19 (d, J = 2Hz, 2H): aromatic H, phenyl; 7.01 (s, 2H): aromatic H, 4-picolyl; 5.2 (s, 1H): OH; 4.15-3.77 (several m, 5 H): P-O-C$\underline{H}_2$-CH_3 and C$\underline{H}$-PO_3Et_2; 3.75 and 3.54 (2d, J = 14 Hz): NH-C$\underline{H}_2$-Py 1.44 and 1.33 (2s, 9H each): phenyl-tert-butyl and pyridyl-tert-butyl; 1.29 and 1.10 (2t, J = 7Hz, 6H): P-O-CH_2-$\underline{CH_3}$

Example 18 - Hydrochloride salt of diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate

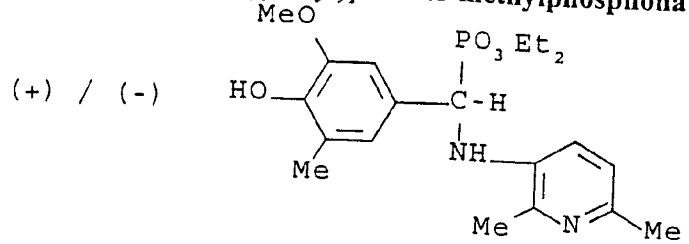


Diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate (4.2g, 9.6mmol) was suspended in 20 ml diethylether and cooled in an ice bath. A solution of HCl in Et_2O (1M, 17.5ml) was added, after stirring for 45 min the mixture was evaporated under reduced pressure until constant weight. An amount of 4.1g (90%) of a yellow solid was obtained.

Elemental analysis: $C_{22}H_{34}ClN_2O_5P$

% Calc.	C 55.87	H 7.25	Cl 7.50N 5.92	P6.55
% Found	C 54.01	H 7.42	Cl 7.54N 5.73	P6.22

Example19 - Enantiomers of diethyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate



Racemic compound (Example 4) was resolved into its two enantiomers by chiral chromatography, using the foillowing conditions:
Column: Chiralpak AD, 250mm x 20mm i.d
Mobile Phase: 85/15 Hexane/Ethanol v/v
Flow Rate: 10ml/min
Detection: UV at 215nm
Sample Concentration: 50mg dissolved in 10ml of 50/50 Hexane/Ethanol v/v
Injection Volume: 500ul

Under these conditions, the first elulting enantiomer peak eluted at 14.7 minutes and the second eluting peak eluted at 18.6 minutes. The two peaks were just baseline resolved. The two peaks were collected as separate fractions over a number of injections. A small sample of each enantiomer fraction was removed for chiral analysis to determine the enantiomeric purity of each fraciton. The HPLC conditions used for this chiral analysis were as follows:
Column: Chiralpak AD, 250mm x 4.6mm i.d
Mobile Phase: 85/15 Hexane/Ethanol v/v
Flow Rate: 1ml/min
Detection: UV at 215nm
Injection Volume: 20ul
Sample Concentration: Unknown - sample of undried peak fraction used.

Under these conditions the main peak of the first eluting enantiomer fraction eluted at 6.95 minutes. No peak due to the minor enantiomer was observed in this fraction. The main peak of the second eluting enantiomer fraction eluted at 6.85 minutes with a small peak due to the minor enantiomer also observed eluting at 7.1 minutes and representing 0.3 % of the total enantiomer peak area.

The remainder of each enantiomer fraction was dried on a rotary evaporator. Each fraction has then been resuspended in a few mls ethanol and transferred to a small

preweighed vial. The samples were blown to dryness under nitrogen at present prior to measurement of their mass spec and optical rotation.

M^+H for each enatiomer = 409.1

First eluting enantiomer: $[\alpha]^D$ at 25°C = +7.93° (c = 1.19% EtOH)

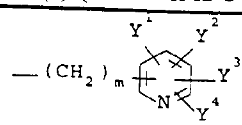
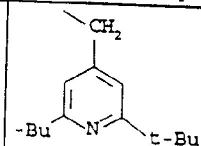
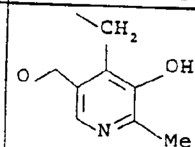
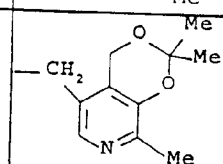
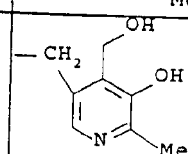
Second eluting enantiomer: $[\alpha]^D$ at 25°C = -8.29° (c = 1.09% EtOH)

Example 20 - Dimethyl α (4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)] amino-methylphosphonate

Dimethyl phosphite (0.4 g, 3.7 mmol) was added to 0.5 g (1.85 mmol) of the crude imine (obtained as described in example 3) and the mixture was heated to 120°C for 2h. The oily reaction mixture was diluted in chloroform and extracted with a saturated bicarbonate solution. The dried organic phase was concentrated and triturated in petroleum ether to remove the excess of dimethyl phosphite. Further purification by column chromatography (SiO_2, 95/5 $CHCl_3$/MeOH) and recrystallization (petroleum ether/CH_2 Cl_2) gave 0.25 g (34%) of a solid, mp = 166-168°C.
IR (KBr) = 3300 cm -1 : NH, 1240: P=O, 1030 : P-O-C

Further compounds of formula (I) were prepared by following procedures anologous to those described in the foregoing examples. The are included in the following Table 1, along with the preceding examples. The left hand column refers to a 'Compound' rather than the Example number, the same compound numbers being then used in the Biological Data section.

Table 1 :Aminophosphonates of formula (I) (where n is 0 and R^1, R^2 are identical)

Cpd No	Ex No	X^1	X^2	X^3	Z		R^1, R^2	mp(°C)
1		OMe	OMe	H	H	3-(2,6-dimethyl)pyridyl	Et	152-154
2	4	OMe	Me	H	H	3-(2,6-dimethyl)pyridyl	Et	145-147
3	3	OMe	Me	H	H	3-(2,6-dimethyl)pyridyl	iPr	159-160
4	1	Me	Me	H	H	3-(2,6-dimethyl)pyridyl	Et	solid
5	2	tBu	Me	H	H	3-(2,6-dimethyl)pyridyl	Et	139-141
6		OEt	Me	H	H	3-(2,6-dimethyl)pyridyl	Et	125-127
7		OEt	Me	H	H	3-(2,6-dimethyl)pyridyl	iPr	145-146
8	17	tBu	tBu	H	H		Et	132-137
9		tBu	tBu	H	H		Et	139-145
10		tBu	tBu	H	H		Et	78-90
11		tBu	tBu	H	H		Et	172-176
12	8	OMe	Me	Me	H	3-(2,6-dimethyl)pyridyl	Et	oil
13	9	OMe	Me	Me	H	3-(2,6-dimethyl)pyridyl	iPr	oil
14	10	OH	Me	Me	H	3-(2,6-dimethyl)pyridyl	Et	141-142
15	11	OH	Me	Me	H	3-(2,6-dimethyl)pyridyl	iPr	168-169
16	12	OH	OMe	Me	H	3-(2,6-dimethyl)pyridyl	Et	134-136
17	13	OH	OMe	Me	H	3-(2,6-dimethyl)pyridyl	iPr	157-159
18	14	OMe	Me	H	H	3-(2,6-dichloro)pyridyl	Et	solid
19	15	OMe	Me	H	H	3-(2,6-dichloro)pyridyl	iPr	124-125
20	16	OMe	OMe	H	H	3-(2,6-dimethoxy)pyridyl	Et	oil
21*	5	OMe	Me	H	H	3-(2,6-dimethyl)pyridyl	iPr	123-124
22*	5	OMe	Me	H	H	3-(2,6-dimethyl)pyridyl	iPr	120-122
23*	19	OMe	Me	H	H	3-(2,6-dimethyl)pyridyl	Et	?
24*	19	OMe	Me	H	H	3-(2,6-dimethyl)pyridyl	Et	?

* Cpd 21 - (+) Enantiomer of Cpd 3; Cpd 22 - (-) Enantiomer of Cpd 3; Cpd 23 - (+) Enantiomer of Cpd 2; Cpd 24 - (-) Enantiomer of Cpd 2

Biological Data

The compounds of formula (I) were assayed for lowering the production of Lp(a) in primary cultures of Cynomolgus hepatocytes.

Assay

Hepatocytes were isolated from livers of adult Cynomolgus monkeys by the two-step collagenase perfusion method according to C. Guguen-Guillouzo and A. Guillouzo "Methods for preparation of adult and fetal hepatocytes" p.1-12 in "Isolated and Cultured Hepatocytes", les editions Inserm Paris and John Libbey Eurotext London (1986).

The viability of cells was determined by Trypan blue staining. The cells were then seeded at a density from 0.7. 10^5 to 1.10^5 viable cells per cm^2 in tissue culture plates in Williams E tissue culture medium containing 10% fetal calf serum. Cells were incubated for 4-6 hours and 24 hours at 37°C in a CO_2 incubator (5% CO_2) in the presence of 20μM of the test compounds dissolved in ethanol. Four to six wells were used for each compound. Nicotinic acid and steroid hormones were used as references to validate the assay system since they are known to decrease Lp(a) in man. Control cells were incubated in the presence of ethanol only.

Results

(a) Lp(a) concentration :

The amount of Lp(a) secreted in culture medium was directly assayed by ELISA using a commercially available kit. Cells were washed and lysed as described by A.L. White et al, Journal of Lipid Research vol 34, p. 509-517, (1993) and the cellular content of Lp(a) was assayed as described above.

Changes in Lp(a) concentration in culture medium are given as the percentage of values measured for the control plates at 24h.

All compounds were tested at 20 μM. Compounds No. 1, 2, 3, 4, 5, 6, 7, 15, 16, 21 and 22 were found to decrease the Lp(a) secretion by 20% to 50% . Compounds 12, 13, 14, 17, 18 and 19 lowered the Lp(a) secretion by 13 to 20%.

(b) *In vivo* Results

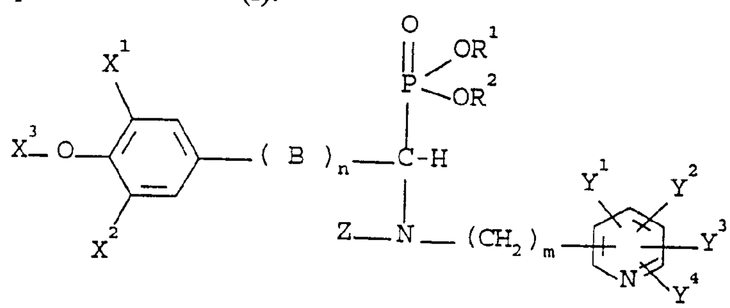
Study Protocol - Male cynomolgus monkeys weighing between 3 and 7 kg were divided into groups of 3 to 4 animals each. Prior to treatment their plasma Lp(a) levels were followed over a two-month period to ascertain a constant baseline value. The Lp(a) values measured at Day -7 and Day -1 were comparable and served as predose values. Test compounds were given orally in gelatin capsules by gavage at the dose of 25 mg/kg/day for 4 weeks and Lp(a) was measured at weekly intervals (Day 7, 14, 21 and 28). At the end of the dosing period, animals were maintained for a treatment free period of 4 weeks, whereupon their plasma Lp(a) levels returned to pretreatment levels. This control provided proof that the decrease in Lp(a) measured was caused by the pharmacological activity of the test compounds.

Results - At Days -7, -1, 7, 14, 21 and 28, after an overnight fast blood samples were collected on EDTA and Lp(a) was measured by the highly sensitive and specific ELISA test. Results (mean of 3-4 values of each group) were expressed as % of predose values. Selected compounds of formula (I) were tested under the experimental conditions to investigate their pharmacological activity in vivo.

Compounds No 2, 3 and 6 were tested at 25mg/kg/day for 28 days and lowered plasma Lp(a) in the range of 15% to 27% (values measured at Day 28, % change from predose values). Compounds 21 and 22 were tested at 50mg/kg/day for 10 days and decreased plasma Lp(a) in the range of 13 to 39% (values measured at Day 10, % change from predose values).

Claims

1. A compound of structure (I):



(I)

in which:
X^1 and X^2, which may be the same or different, are H, a straight or branched $C_{(1-8)}$alkyl or $C_{(1-8)}$alkoxy group, a hydroxy group or a nitro group;
X^3 is H, a $C_{(1-4)}$alkyl group, X^3O and one of the two other substituents X^1 or X^2 may form a $C_{(1-4)}$alkylidene dioxy ring;
R^1 and R^2, which may be the same or different, are H, a straight or branched $C_{(1-6)}$alkyl group;
B is CH_2, CH_2-CH_2 or CH=CH;
n is zero or 1;
Z is H, or a straight or branched $C_{(1-8)}$alkyl group;
m is 0 or an integer from 1 to 5; and
Y^1, Y^2, Y^3 and Y^4, which may be the same or different, are H, a straight or branched $C_{(1-8)}$alkyl or $C_{(1-8)}$alkoxy group, a cyano, trifluoromethyl, nitro, hydroxy, hydroxymethyl, $C_{(1-4)}$ alkoxymethyl, amino, $C_{(1-4)}$alkylamino, $C_{(1-4)}$dialkylamino group, a halogen atom (F, Cl, Br, I), or any two adjacent Y^1, Y^2, Y^3 and Y^4 may form an optionally substituted $C_{(1-6)}$alkylidene or $C_{(1-4)}$alkylidenedioxy ring, with the proviso that at least two of the Y^1, Y^2, Y^3 and Y^4 groups are not H;
or a pharmaceutically acceptable salt thereof.

2. A compound as claimed in claim 1 in which X^1 is H, hydroxy, $C_{(1-4)}$alkyl or $C_{(1-4)}$alkoxy.

3. A compound as claimed in claim 1 or 2 in which X^2 is $C_{(1-4)}$alkyl or $C_{(1-4)}$alkoxy.

4. A compound as claimed in any one of claims 1 to 3 in which X^1 and X^2 is each $C_{(1-4)}$alkyl or $C_{(1-4)}$alkoxy; or or one of X^1 and X^2 is $C_{(1-4)}$alkyl and the other is

$C_{(1-4)}$alkoxy or $C_{(1-3)}$alkyl; or X^1 is hydroxy and X^2 is $C_{(1-4)}$alkyl or $C_{(1-4)}$alkoxy.

5. A compound as claimed in any one of claims 1 to 4 in which X^1 and X^2 are methoxy and methoxy, methoxy and methyl, ethoxy and methyl, methyl or t-butyl and methyl, ethoxy and ethoxy, hydroxy and methyl, and hydroxy and methoxy, respectively.

6. A compound as claimed in any one of claims 1 to 5 in which X^3 is hydrogen or methyl.

7. A compound as claimed in any one of claims 1 to 6 in which $(B)_n$ is a direct bond.

8. A compound as claimed in any one of claims 1 to 7 in which Z is hydrogen.

9. A compound as claimed in any one of claims 1 to 8 in which Y^1 and Y^2 is each methyl and Y^3 and Y^4 is each hydrogen..

10. A compound as claimed in claim 9 in which Y^1 and Y^2 are 2,6-substituents of the pyridyl ring.

11. A compound as claimed in any one of claims 1 to 10 in which the pyridyl ring is attached by the ring carbon β- to the nitrogen (3/5-pyridyl).

12. A compound as claimed in any one of claims 1 to 10 in which m is zero.

13. A compound of formula (I) as defined in claim 1 selected from:
diethyl α-(4-hydroxy-3,5-dimethoxyphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate;
diethyl α-(4-hydroxy-3,5-dimethylphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate;
diethyl α-(3-tert-butyl-4-hydroxy-3-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate;
diethyl α-(3-ethoxy-4-hydroxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate;
diisopropyl α-(3-ethoxy-4-hydroxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate;

diethyl α-(3,5-di-tert-butyl-4-hydroxyphenyl)-N-[4-(2,6-di-tert-butylpicolyl)]-aminomethylphosphonate;
diethyl α-(3,5-di-tert-butyl-4-hydroxyphenyl)-N-[4-(3-hydroxy-5-hydroxymethyl-2-methylpicolyl)]-aminomethylphosphonate;
diethyl α-(3,5-di-tert-butyl-4-hydroxyphenyl)-N-[5-(3,4-O-isopropylidene-3-hydroxy-4-hydroxymethyl-2-methylpicolyl)]-aminomethylphosphonate;
diethyl α-(3,5-di-tert-butyl-4-hydroxyphenyl)-N-[5-(3-hydroxy-4-hydroxymethyl-2-methylpicolyl)]-aminomethylphosphonate;
diethyl α-(3,4-dimethoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate;
diisopropylα-(3,4-dimethoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate;
diethyl α-(3-hydroxy-4-methoxy-5-methylphenyl)- N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate;
diisopropyl α-(3-hydroxy-4-methoxy-5-methylphenyl)- N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate;
diethyl α-(4,5-dimethoxy-3-hydroxyphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate;
diisopropyl α-(4,5-dimethoxy-3-hydroxyphenyl)-N-[3-(2,6-dimethylpyridyl)]-amino-methylphosphonate;
diethyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dichloropyridyl)]-amino-methylphosphonate;
diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dichloropyridyl)]-amino-methylphosphonate; and
diethyl α-(3,5-dimethoxy-4-hydroxyphenyl)-N-[3-(2,6-dimethoxypyridyl)]-amino-methylphosphonate; or
a pharmaceutically acceptable salt thereof.

14. A compound of formula (I) as defined in claim 1 selected from:
diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate;
(+)diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate;
(-)diisopropyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate; or
a pharmaceutically acceptable salt thereof, in particular the hydrochloride salt.

15. A compound of formula (I) as defined in claim 1 selected from:

diethyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate;
(+)diethyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate; and
(-)diethyl α-(4-hydroxy-3-methoxy-5-methylphenyl)-N-[3-(2,6-dimethylpyridyl)]-aminomethylphosphonate; or
a pharmaceutically acceptable salt thereof, in particular the hydrochloride salt.

16. A pharmaceutical composition comprising a compound of formula (I) as defined in claim 1 and a pharmaceutically acceptable excipient thereof.

17. A compound of formula (I) as defined in claim 1, or a pharmaceutically acceptable salt thereof, for use in therapy.

18. The use of a compound of formula (I) as defined in claim 1, or a pharmaceutically acceptable salt thereof, for the manufacture of a medicament for use in decreasing plasma and tissue lipoprotein(a) levels.

19. A use of a compound of formula (I) as claimed in claim 18, for the manufacture of a medicament for the treatment of thrombosis by decreasing plasma lipoprotein(a) levels.

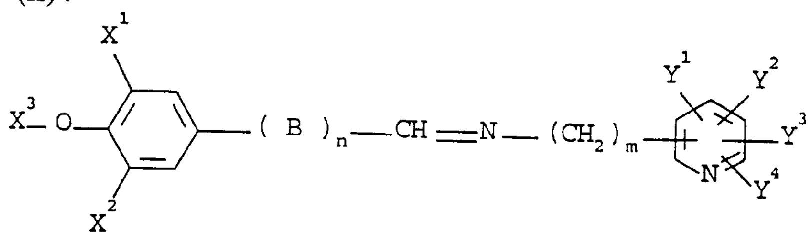
20. A use of a compound of formula (I) as claimed in claim 18, for the manufacture of a medicament for the treatment of restenosis following angioplasty by decreasing plasma lipoprotein(a) levels.

21. A use of a compound of formula (I) as claimed in claim 18, for the manufacture of a medicament for the treatment of atherosclerosis by decreasing plasma lipoprotein(a) levels.

22. A method of treating a disease associated with elevated plasma and tissue lipoprotein(a) levels which method comprises administering to a patient in need thereof a therapeutically effective amount of a compound of formula (I) as defined in claim 1, or a pharmaceutically acceptable salt thereof.

23. A process for preparing a compound of formula (I) as defined in claim 1 which process comprises:

(a) for compounds of formula (I) in which Z is hydrogen, treating an imine of formula (II) :



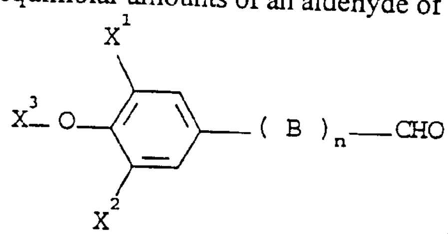
(II)

in which X^1, X^2, X^3, B, n, m, Y^1, Y^2, Y^3 and Y^4 are as defined in claim 1; with a dialkyl phosphite of formula (III):

H-PO(OR^1)(OR^2) (III)

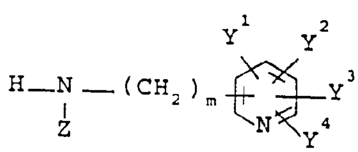
in which R^1 and R^2 are as defined in claim 1; or a trialkyl silyl or metal derivative thereof;

(b) reacting together equimolar amounts of an aldehyde of formula (IV):



(IV)

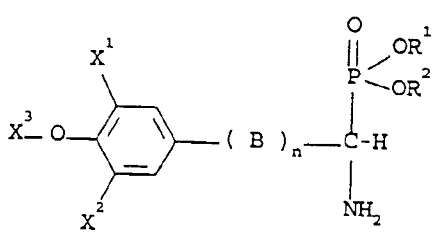
in which X^1, X^2, X^3, B and n are as defined in claim 1;
an amine of formula (V):



(V)

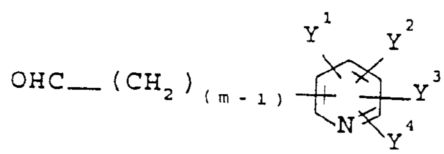
in which Z, m, Y^1, Y^2, Y^3 and Y^4 are as previously defined; and a dialkyl phosphite of formula (III; or

(c) for compounds of formula (I) in which m is not zero, treating a compound of formula (VI)



(VI)

in which X^1, X^2, X^3, B and n are as defined in claim 1, with an aldehyde of formula (VII):



(VII)

in which m is an integer from 1 to 5 and Y^1, Y^2, Y^3 and Y^4 are as defined in claim 1; under reductive amination conditions.

INTERNATIONAL SEARCH REPORT

Inte   .onal Application No
PCT/EP 97/07161

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6   C07F9/58   A61K31/675

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6   C07F   A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 559 079 A (SYMPHAR S.A.) 8 September 1993 cited in the application see the whole document ---	1-23
A	EP 0 703 239 A (HOECHST AG) 27 March 1996 see the whole document ---	1-23
P,Y	WO 97 02037 A (SYMPHAR S.A.) 23 January 1997 cited in the application see the whole document -----	1-23

☐ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 March 1998	31/03/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Beslier, L

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inter nal Application No
PCT/EP 97/07161

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